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Evaluation of microbial consortia against Okra root rot [*Macrophomina phaseolina* (Tassi) Goid.]

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ABSTRACT

Okra [*Abelmoschus esculentus* (L.) Moench] is the only vegetable crop of significance in the *Malvaceae* family and is very popular in the Indo-Pak subcontinent. In India, it ranks first in its consumption. The crop was found to suffer from stem and root rot disease in severe form in many region of Gujarat state during *Kharif*, 2021. So for its management different biocontrol agents evaluated under field condition, recorded minimum disease incidence 17.88 % was recorded in *T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM which was found at par with *T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM with 22.07 per cent disease incidence and the highest yield (100.99 q/ha) was obtained in *T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM, which was statically at par with *T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM (94.65 q/ha).

KEYWORDS

Okra, Root Rot, Biocontrol Agents, *Macrophomina phaseolina*

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Okra [*Abelmoschus esculentus* (L.) Moench] is the only vegetable crop of significance in the *Malvaceae* family and is very popular in the Indo-Pak subcontinent. In India, it ranks number one in its consumption. Okra is attacked by several fungal pathogens, which not only reduces the potency of seed, but also degrades the health beneficial and nutritional quality components. The most serious fungal diseases of okra are damping off and root rot (*Macrophomina phaseolina, Pythium aphanidermatum* and *Rhizoctonia solani*), vascular wilt (*Fusarium oxysporum*), *Cercospora* blight (*Cercospora abelmoschus, Cercospora malayensis*) and powdery mildew (*Erysiphe cichoracearum, Oidium abelmoschi*) (Anonymous, 2013). *M. phaseolina* is a necrotrophic plant pathogen, with heterogeneous host specificity (Mayek *et al.*, 2001). Rangaswami (1993) described the pathogen *Macrophomina phaseolina* affects the fibrovascular system of the roots and basal stem of its host, impeding the transport of water and nutrients to the upper parts of the plant.

Roots of infected plants rot, plants wilt, and ultimately die when the disease reach at advance stages. The disease symptom starts initially with yellowing and drooping of the leaves and later infected leaves fall off pre-maturely and the plant dies within a short period. The infected plant shows dark brown lesions on the stem at ground level and bark shows shredding symptom. The affected plants can be easily pulled out leaving dried, rotten root portions in the ground. Hence, different biocontrol agents tested against *Macrophomina phaseolina.*

Materials and Methods

A field trial was conducted at research farm, Department of Plant Pathology, JAU, Junagadh in *Kharif,* 2021 to study efficacy of various biocontrol agent for managing root rot of okra caused by *M. phaseolina* (table 1). The trials were arranged in randomized block design with three replications. The seeds of okra variety 'Gujarat Junagadh Okra-3' was sown at the rate of 10 kg seed per hectare on onset of monsoon. The seeds were sown at 60 cm × 30 cm distance in each of the gross plot size of 4.50 m × 3.60 m and net plot size 3.90 m × 2.40 m manually in fertilized (150:50:50 NPK kg/ha) soil. All agronomic practices were followed as and when required.

The inoculum of *M. phaseolina* prepared on half cooked sorghum grains as stated earlier was incorporated one week before sowing in the soil at the rate of 100 kg per ha. The required quantity of talc based biocontrol agents was incorporated in the soil at the time of sowing. The symptoms of root rot developed if any were observed periodically and the disease incidence was worked out in each treatment for effectiveness of biocontrol agents under field condition using following formula. The disease infected plants was counted and Percent Disease Incidence (PDI) was calculated by using the following formula (Wheeler, 1969). The symptoms of *M. phaseolina* disease of okra were studied and expression of the characteristic symptoms and variation if any was recorded.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

The biochemical parameters were determined from each treatment adopting standard methods.

Total Sugar Estimation

One gram root of okra plant were extracted with 10 ml of 80 % methanol and centrifuged it, then collect supernatant. Extraction procedure was repeated 4 times with 80% methanol and supernatant was collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80 % methanol. 0.1 ml aliquot from the extract was pipette into separate test tubes and the tubes were placed in a boiling water bath to evaporate the methanol. One ml of Millipore water and 1ml of 5 % phenol was added in each test tube. Then 5 ml of sulphuric acid was added. The tubes were allowed to cool in ice-bath for 10-15 minutes. The intensity of colour was read at 490 nm on spectrophotometer (Dubois *et al.,* 1956). A standard curve was prepared using 10 mg glucose per 100 ml distilled water and soluble sugar content expressed as % and after calculated as mg/g.

$$\text{Soluble Sugar} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total Volume} \times 100 \times 10^{-6}}{\text{Aliquote} \times \text{sample taken}}$$

Total Phenol Estimation

Suitable aliquot (0.1ml) was taken from methanol extract prepared for total phenol analysis and evaporated to dryness in water bath. One ml of Millipore water in each test tube and 0.5 ml of folin ciocalteu's phenol reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was read at 760 nm against a reagent blank (Snell and Snell, 1953). A standard graph was prepared using pyrocatachol ranging between 10-15 µg concentrations. The amount of phenols present in the sample was calculated as.

$$\text{Soluble phenol} = \frac{\text{Graph factor} \times \text{O.D.} \times \text{Total Volume} \times 100 \times 10^{-6}}{\text{Aliquote} \times \text{sample taken}}$$

Total Protein Estimation

Total protein concentration in plant extract was checked by Folin-Lowry's method (Lowry *et al.,* 1951). For estimation of protein 200 µl of plant extract, 800 µl of distilled water and 5 ml of alkaline copper reagent were mixed together and incubate for 10 minutes at room temperature.

Table 1. List of different biological control agents against root rot of okra *in vivo*

S. N.	Biocontrol Agents
1.	Soil appl. of Trichoderma harzianum @ 2.5 kg/ha mixed in 300 kg FYM/ha at the time of sowing
2.	Soil appl. of Trichoderma viride @ 2.5 kg/ha mixed in 300 kg FYM at the time of sowing
3.	Soil appl. of Pseudomonas fluorescens @ 2.5 kg/ha mixed in 300 kg FYM at the time of sowing
4.	Soil appl. of Trichoderma harzianum @ 5 kg/ha mixed in 300 kg FYM at the time of sowing
5.	Soil appl. of Trichoderma viride @ 5 kg/ha mixed in 300 kg FYM at the time of sowing
6.	Soil appl. of Pseudomonas fluorescens @ 5 kg/ha mixed in 300 kg FYM at the time of sowing
7.	Soil appl. of Trichoderma harzianum @ 2.5 kg/ha + Pseudomonas fluorescens @ 2.5 kg/ha + 300 kg FYM at the time of sowing
8.	Soil appl. of Trichoderma viride @ kg/ha + Pseudomonas fluorescens @ 2.5 kg/ha + 300 kg FYM at the time of sowing
9.	No any biocontrol agent (absolute control)
	N.B.: Spore mass of bioagents having minimum cfu (colony forming unit) 2 ⟦×10⟧^7/g and ⟦1x10⟧^8/g for fungus and bacterium, respectively.

Table 2. Field evaluation of biocontrol agents against root rot (*M. phaseolina*) of okra

Tr.	Treatment	Disease Incidence (%)	Yield (Q/ha)	Total Sugar (Mg/g)	Total Phenol (Mg/g)	Total Protein (Mg/g)
T1	Soil appl. of *T. harzianum* @ 2.5 kg/ha mixed in 300 kg FYM/ha at the time of sowing	33.41 (30.33)	77.33	6.20	1.24	8.28
T2	Soil appl. of *T. viride* @ 2.5 kg/ha mixed in 300 kg FYM at the time of sowing	32.02 (28.11)	82.82	6.31	1.31	8.58
T3	Soil appl. of *P. fluorescens* @ 2.5 kg/ha mixed in 300 kg FYM at the time of sowing	34.30 (31.76)	75.78	6.14	1.11	8.18
T4	Soil appl. of *T harzianum* @ 5 kg/ha mixed in 300 kg FYM at the time of sowing	31.48 (27.27)	89.12	6.36	1.27	9.29
T5	Soil appl. of *T viride* @ 5 kg/ha mixed in 300 kg FYM at the time of sowing	30.85 (26.29)	92.76	6.44	1.34	9.71
T6	Soil appl. of *P. fluorescens* @ 5 kg/ha mixed in 300 kg FYM at the time of sowing	32.38 (28.69)	83.74	6.29	1.25	8.38
T7	Soil appl. of *T.harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha mixed in 300 kg FYM at the time of sowing	28.02 (22.07)	94.65	6.65	1.54	9.55
T8	Soil appl. of *T. viride @ 2.5* kg/ha + *P. fluorescens* @ 2.5 *kg/*ha mixed in 300 kg FYM at the time of sowing	25.02 (17.88)	100.99	6.70	1.56	9.84
T9	Control	48.46 (56.02)	58.09	5.38	1.04	6.88
	S.Em.±	1.93	4.48	0.13	0.01	0.17
	C.D. at 5 %	5.64	13.43	0.38	0.05	0.52
	C.V. %	10.19	9.25	3.62	2.59	3.55

500 µl of Folin-Ciocalteu reagent was added and incubate for 30 minutes in dark. The intensity of blue color was measured at 660 nm using spectrophotometer. Bovine serum albumin was used as an internal standard.

The protein content was calculated as stated below and value expressed as $mg.g^{-1}$.

$$\textbf{Soluble Protein (mg.g}^{-1}\textbf{)} = \frac{\textbf{Graph factor} \times \textbf{O.D.} \times \textbf{Total Volume} \times 100 \times 10^{-6}}{\textbf{Aliquote} \times \textbf{sample weight}}$$

Results and Discussion

Field evaluation of biocontrol agents against root rot of okra conducted during *Kharif*, 2021 experimental farm of Department of Plant Pathology, JAU, Junagadh, Gujarat. Different biocontrol agents mixed with FYM were evaluated under field condition. The experiment was laid out in completely randomized design with three repetitions. Total nine treatments including control were applied as treatment. Okra variety Gujarat Junagadh Okra-3 seeds were sown at 60 cm × 30 cm distance in each of the gross plot size of 4.50 m × 3.60 m and net plot size 3.90 m × 2.40 m required quantity of biocontrol agents were incorporated in each treatment before sowing. Root rot incidence observations were recorded periodically and biochemical analysis was carried out at 40 days after sowing. The data are presented in table 2.

Percent Disease Incidence

Perusal of data presented in table 2 revealed that all the treatments significantly reducing root rot incidence as compared to the control. Minimum disease incidence (17.88 %) was recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) which was found at par with T7 (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) with 22.07 per cent disease incidence and T5 (*T. viride* @ 5 kg/ha + 300 kg FYM) showed 26.29 per cent disease incidence. T4 (*T. harzianum* @ 5 kg/ha + 300 kg FYM) exhibited 27.27 per cent disease incidence and was found at par with T2 (*T. viride* @ 2.5 kg/ha + 300 kg FYM) which recorded 28.11 per cent disease incidence. Next was T6 (*P. fluorescens* @ 5 kg/ha + 300 kg FYM), T1 (*T. harzianum* @ 2.5 kg/ha + 300 kg FYM) and T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) were exhibited 28.69, 30.33 and 31.76 percent disease incidence, whereas control showed maximum disease incidence which was highest among all (56.02 %).

Yield

The yield was also significantly higher in all treatments as compared to control. The highest yield (100.99 q/ha) was obtained in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM), which was statically at par with T7 (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (94.65 q/ha).

Whereas, next best treatments were T5 (*T. viride* @ 5 kg/ha + 300 kg FYM), T4 (*T. harzianum* @ 5 kg/ha + 300 kg FYM), T6 (*P. fluorescens* @ 5 kg/ha + 300 kg FYM) and T2 (*T. viride* @ 2.5 kg/ha + 300 kg FYM) was recorded 92.76, 89.12, 83.74 and 82.82 q/ha okra fruit yield, respectively, whereas, control plant showed minimum yield (58.09 q/ha). These result revealed that significantly higher yield and minimum disease incidence were obtained in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) and T7 (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) so these could be recommended for controlling disease under field condition.

Total Sugar

The results presented in table 2 revealed that in different treatment, highest sugar content was recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (6.70 mg/g) and it was at par with (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (6.65 mg/g) and T5 (*T. viride* @ 5 kg/ha + 300 kg FYM) (6.44 mg/g). Whereas, other treatments T4 (*T. harzianum* @ 5 kg/ha + 300 kg FYM), T6 (*P. fluorescens* @ 5 kg/ha + 300 kg FYM) and T2 (*T. viride* @ 2.5 kg/ha + 300 kg FYM) was recorded total sugar 6.36, 6.29 and 6.31 mg/g total sugar, respectively. Whereas, lowest sugar content was recorded in T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (6.14 mg/g). Infected plant recorded 5.38 mg/g total sugar.

Total Phenol

The results presented in table 2 revealed that highest phenol content was recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (1.56 mg/g) and it was at par with T7 (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (1.54 mg/g) and it was followed by T5 (*T. viride* @ 5 kg/ha + 300 kg FYM) (1.34 mg/g). Whereas, other treatments T2 (*T. viride* @ 2.5 kg/ha + 300 kg FYM), T4 (*T. harzianum* @ 5 kg/ha + 300 kg FYM) and T6 (*P. fluorescens* @ 5 kg/ha + 300 kg FYM) was recorded total phenol 1.31, 1.27 and 1.25 mg/g, respectively. Whereas, lowest phenol content was recorded in T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (1.11 mg/g). Infected plant showed 1.04 mg/g phenol content.

Total Protein

The results presented in table 2 revealed that highest protein content was recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (9.84 mg/g) and it was at par with T7 (*T. harzianum* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (9.55 mg/g) and T5 (*T. viride* @ 5 kg/ha + 300 kg FYM) (9.71 mg/g). Whereas, other treatments T4 (*T. harzianum* @ 5 kg/ha + 300 kg FYM), T2 (*T. viride* @ 2.5 kg/ha + 300 kg FYM) and T6 (*P. fluorescens* @ 5 kg/ha + 300 kg FYM) was recorded total protein contain 9.29, 8.58 and 8.38 mg/g, respectively. Lowest protein content was recorded in T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (8.18 mg/g). Infected plant showed 6.88 mg/g total protein content.

Our result was also in line with finding of who (Aravind, 2017) tested five different bioagents against *M. phaseolina,* incident of root and collar rot of okra, revealed that *T. viride* was the most effective with highest growth inhibition followed by *T. harzianum*. Madhi *et al.* (2020) revealed that *P. fluorescens* had a clear ability in increasing the germination percentage of okra seeds and reduction in damping-off (*M. phaseolina*, *F. solani* and *R. solani*) its seedling. While, in case of biochemical parameters these results are in agreement with the finding of **(**Pancham *et al.*, 2016) reported the reduction in the contents of total sugars, reducing and non-reducing sugars in the roots of dry root rot disease caused by *M. phaseolina* in groundnut.

Aravind and Brahmbhatt (2018) carried out biochemical analysis of the resistant and susceptible genotypes of okra against root and collar rot caused by *M. phaseolina* and revealed that the total soluble sugar, reducing sugar and non-reducing sugar decreased following inoculation by the pathogen. Reddy and Sireesha *et al.* (2013) reported that healthy plants recorded higher amount of sugars than diseased plants. The reduction in sugar content after infection may due to rapid hydrolysis of sugars during pathogenesis through enzymes (hydrolases) secreted by pathogens and subsequent utilization by pathogens for their development. These results are in agreement with the finding of **(**Kumar *et al.*, 2019) reported that there was a significant increase in phenol content of mung bean roots due to dry root rot caused by *M. phaseolina* as compared healthy roots after 45 days of sowing.

Bhaskaran *et al*. (1975) reported accumulation of phenolic compound around the infection site as immediate host response. Prasad and Reddy (1987) reported that all the plants containing certain amount of phenols, but level of phenol content increased whenever the plants gets infected. Similarly, (Aravind and Brahmbhatt, 2018) carried out biochemical analysis of the resistant and susceptible genotypes of okra against root and collar rot caused by *M. phaseolina* and revealed that the total protein contain decreased following inoculation by the pathogen. Kumar *et al*. (2019) at Bikaner (Rajasthan) carried out studies on changes in total protein content in dry root rot (*M. phaseolina*) infected plants of mung bean and reported that there was a significant decrease in soluble protein content in diseased roots as compared to healthy roots in all the tested varieties.

Summary and Conclusion

It is concluded that different eight biocontrol agents evaluated under field condition and found minimum disease incidence (17.88 %) was recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) and the highest yield (100.99 q/ha). Whereas control showed maximum disease incidence which was highest among all (56.02 %) and control plant showed minimum yield (58.09 q/ha). Biochemical analysis was carried out at 40 days after sowing and recorded total sugar content decreased after infection of *M. phaseolina* as compared to treatment plant. In different treatment, highest sugar content recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (6.70 mg/g). Whereas, lowest phenol content was recorded in T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (6.14 mg/g).

The results revealed that total phenol content increased after infection of *M. phaseolina* as compared to treatment plant. In different treatment, highest phenol content recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (1.56 mg/g). Whereas, lowest phenol content was recorded in T3 (*P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (1.11 mg/g). Infected plant showed 1.04 mg/g phenol content. Total protein content decreased after infection of *M. phaseolina* as compared to treatment plant.

In different treatment, highest protein content recorded in T8 (*T. viride* @ 2.5 kg/ha + *P. fluorescens* @ 2.5 kg/ha + 300 kg FYM) (9.84 mg/g) and lowest protein content was recorded in T1 (*T. harzianum* @ 2.5 kg/ha + 300 kg FYM) (8.18 mg/g).

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